

Methods to Characterize Ricin for the Development of Reference Materials for Testing Detection Devices

Ricin is a highly toxic protein that is found in castor beans obtained from the tropical plant Ricinus communis. Because of the ease of isolation and high toxicity, ricin is recognized as a major biological terrorism agent. NIST is addressing the technical challenges in accurately measuring ricin in preparation for the development of a Reference Material to be used in the detection and quantification of ricin.

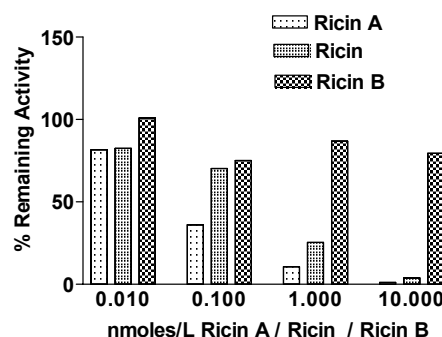
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Ricin can be easily isolated in abundance from the pulp of the tropical plant *Ricinus communis*. There are three routes of exposure to ricin: inhalation, ingestion, and injection; the latter is the most lethal. Estimated ricin doses for human lethality (LD₅₀ values) are 3 µg/kg body weight by inhalation and 30 µg/kg body weight by ingestion. Based on these estimates, 1 kg of ricin evenly spread could cause the loss of more than 2 million human lives. The pulp remaining after removal of the oil from castor beans is a major source of ricin which constitutes ≈ 5% of the castor bean protein.

NIST is addressing the technical hurdles in accurately measuring ricin by investigating various methods for the biochemical characterization of ricin. This will allow for the development of a ricin Reference Material that can be used for the detection, identification, and quantification of ricin. We have characterized the purity and the subunit composition of ricin by using sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Protein concentration has been accurately determined by amino acid analysis, and these results have been compared with those obtained by a method commonly used to determine protein concentration, namely the Lowry method that uses bovine serum albumin and ovalbumin as standards.

We have also implemented a sensitive enzymatic assay for the inhibition of protein synthesis based on the inhibition of [³⁵S]-labeled methionine incorporation into the protein luciferase. In addition, we have carried out an immunoassay for the ricin toxin using flow cytometry. Finally, we isolated the genomic DNA from two varieties of castor beans and developed a PCR-based assay for the detection of the ricin coding sequence.

Effect of ricin A, ricin B, and ricin on protein synthesis



By implementing the techniques described here, NIST is now in a position to enable reproducible and accurate measurements in this field and to develop a Reference Material for devices that detect and measure ricin.

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